Original Article

ABH Secretor Status in the Thai Population

Sirapath Peter Kong, Pimol Chiewsilp, Jintana Tubrod and Pajaree Deesin
National Blood Centre, The Thai Red Cross Society

Abstract:

ABO blood groups and ABH saliva secretion were investigated in the Thai population. A total of 206 blood donors and 2 families, of 3 and 4 members, respectively, were subjected to the study at National Blood Centre, The Thai Red Cross Society. The study was conducted from July 22nd to August 26th, 2016. ABO blood grouping was examined by both cell and serum grouping. Saliva from blood donors was collected and hemagglutination inhibition test (HAI) was used to detect the secretor status. It was found that the gene frequencies of O, A and B were 0.646, 0.142 and 0.212, respectively. The frequencies of Se and se genes were found to be 0.4603 and 0.5397, respectively. It was concluded that among the Thai population, 70.87% were secretors and 29.13% were non-secretors, which was similar to the studies in other populations. The frequencies of secretor status in different ABO blood groups were 74.42% in O, 64.29% in A, 74.63% in B and 45.45% in AB, and the frequencies of secretor status in men and women were 68.00% and 73.58%, respectively. However, there were no significant differences in the secretor status among genders and in blood groups O, A and B ($p > 0.05$), though there were significant differences in the secretor status when comparing blood group AB with blood groups O and B ($p < 0.05$) but no significant differences when comparing with blood group A ($p > 0.05$). The family studies have clearly shown the genetic heritage of Se and se genes in the first family, while in the second family, everyone was an ABH secretor. In conclusion, the frequency of the ABH secretors in the Thai population was comparable with other populations and the detection of saliva ABH substances was able to confirm the obscure ABO blood group determination among the ABH secretors.

Keywords : ● *ABH secretors* ● *ABO blood groups* ● *Saliva hemagglutination inhibition test* ● *Thai population*

Thai J Hematol Transfus Med 2016;26:199-205.

นิพนธ์ต้นฉบับ

สภาวะการหลั่งสาร ABH ในประชากรไทย

สิรพัชร์ ปีเตอร์ คอง พิมล เชี่ยวศิลป์ จินตนา ทับรอด และ ปาจรีย์ ดีสิน
ศูนย์บริการโลหิตแห่งชาติ สภากาชาดไทย

บทคัดย่อ

ได้ทำการศึกษาหมู่โลหิต ABO และการหลั่งสาร ABH ในประชากรไทยจำนวน 206 คน รวมทั้งศึกษา 2 ครอบครัวที่มีสมาชิก 3 และ 4 คนตามลำดับ ณ ศูนย์บริการโลหิตแห่งชาติ สภากาชาดไทย ระหว่างวันที่ 22 กรกฎาคม ถึง 26 สิงหาคม 2559 วิธีการทดสอบหมู่โลหิต ตรวจทั้ง cell และ serum grouping สำหรับการตรวจสาร ABH ในน้ำลาย ใช้วิธี hemagglutination inhibition (HAI) ผลการศึกษา พบว่าความถี่ของยีน O, A และ B เท่ากับ 0.646, 0.142 และ 0.212 ตามลำดับ ความถี่ของยีน Se และ se

Received 26 August 2016 Accepted 12 September 2016

Requests for reprints should be addressed to Pimol Chiewsilp, MD., National Blood Centre, The Thai Red Cross Society, Patumwan Dirst., Bangkok 10330 E-mail : pimol_c@yahoo.com

เท่ากับ 0.4603, 0.5397 ตามลำดับ สามารถสรุปได้ว่าคนไทยเป็น secretor ร้อยละ 70.87 และเป็น non secretor ร้อยละ 29.13 ซึ่งใกล้เคียงกับที่พบรายงานในประชากรอื่นๆ ความถี่ของผู้ที่หลั่งสาร ABH แยกตามหมู่โลหิตเท่ากับร้อยละ 74.42 ในหมู่ O ร้อยละ 64.29 ในหมู่ A ร้อยละ 74.63 ในหมู่ B และร้อยละ 45.45 ในหมู่ AB ความถี่ของผู้หลั่งสาร ABH ในผู้ชายและผู้หญิงเท่ากับร้อยละ 68.00 และร้อยละ 73.58 ตามลำดับ อย่างไรก็ดีความแตกต่างทั้งในระหว่างเพศและหมู่โลหิต O, A และ B ของผู้หลั่งสาร ไม่มีนัยสำคัญทางสถิติ ($p > 0.05$) และถึงแม้ว่าเมื่อเปรียบเทียบผู้หลั่งสารในหมู่โลหิต AB กับหมู่โลหิต O และ B มีความแตกต่างอย่างมีนัยสำคัญ ($p < 0.05$) แต่เมื่อเปรียบเทียบผู้หลั่งสารในหมู่โลหิต AB กับหมู่โลหิต A ไม่มีความแตกต่างอย่างมีนัยสำคัญ ($p > 0.05$) การถ่ายทอดยีน Se และ se สามารถเห็นได้ชัดเจนใน 1 ครอบครัว ส่วนอีกครอบครัวพบว่าเป็น ABH secretor ทุกคน โดยสรุปการศึกษานี้แสดงว่าสภาวะการหลั่งสาร ABH ในน้ำลายในประชากรไทย มีความถี่เช่นเดียวกับประชากรอื่นๆ และสามารถใช้การตรวจสาร ABH ในน้ำลาย เพื่อยืนยันหมู่โลหิต ABO ในกลุ่ม secretors ที่การตรวจปกติให้ผลไม่ชัดเจน

คำสำคัญ : ● *ABH secretors* ● *ABO blood groups* ● *Saliva hemagglutination inhibition test* ● *Thai population*

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต 2559;26:199-205.

Introduction

The precursor of the ABO blood group antigens is known as the H substance. Absence of the H substance means that both A and B antigens cannot be synthesised. People with Bombay (Oh) phenotype do not express H antigen on their red blood cells (RBC) and usually acquire naturally occurring anti-H. This poses a problem in blood transfusion because if a patient with Bombay phenotype receives blood containing H antigen on RBC, an acute hemolytic transfusion reaction may occur due to reaction of H antigen and anti-H. Therefore, it is important that patients with Bombay phenotype receive blood only from other Bombay (Oh) phenotype donors.[1]

The soluble form of H antigen is regulated by *FUT1(H)* and *FUT2(Se)* genes, on 19q13.3.[1] *H* gene encodes for fucosyltransferase 1. Both Bombay and para-Bombay phenotypes are caused by a point mutation in the *H* gene, which leads to gene inactivation. If both copies of *H* genes are inactivated (*hh*), fucosyltransferase 1 cannot be expressed, which results in no production of H antigen. Additionally, *Se* gene indirectly encodes for soluble form of the H antigen. As *Se* gene is dominant, H antigen secretor only contain at least 1 copy of functioning gene (*Sese* or *SeSe*), while a non-secretor (*sese*) does not have the functional gene to produce soluble H antigen.[1]

Another gene that is closely linked to the *H* and *Se* genes is the *FUT3(Le)* gene. *Le* gene adds fucose to type 1 precursor substance to make Le^a antigen. When *Se* gene is active, it adds another fucose onto the Le^a antigen, resulting in Le^b antigen. This means that an individual with active *Le* gene but inactive *Se* gene will have Le^a antigen. Likewise, an individual with both active *Le* and *Se* genes will have Le^b antigen. Inactivated *Le* gene will neither have the Le^a or Le^b antigen.[2]

Additionally, *Se* gene is responsible for the ABH secretion. ABH substances are secreted via various body fluids including saliva, sweat, tears and semen.[2] Secretors secrete ABH substances corresponding to their ABO blood types.[3]

Even though monoclonal typing reagents have been developed and advanced to have high potency, there are still some problems in identifying blood groups in most rare cases. Despite the fact that DNA technology can be used to detect the blood group with great accuracy[4], it poses many problems due to the expenses and the processing time. This means not all hospitals are able to afford such technology. Hemagglutination inhibition test (HAI) can be used as an alternative regarding the identification of blood groups. HAI detects ABH substances in the saliva and this can determine the blood group providing that

the person is a secretor. Approximately 70-80% of the populations were secretors.[1,3,5-7] However, there is no published data regarding ABH secretors in the Thai population. Therefore, this study was aimed to determine the ABH secretor status in the Thai population.

Materials and Methods

Saliva sample collection

Approximately 5 mL saliva was collected from 206 blood donors at National Blood Centre, The Thai Red Cross Society. Saliva was transferred to sterile test tubes and kept in a boiling water bath for 10 minutes to denature salivary and bacterial enzymes. The saliva was then centrifuged and the clear supernatant was transferred to sterile test tubes. Equal volume of saline was added, then mixed well and kept in the freezer at -20°C until they were tested.[8] The first time donors were excluded, since they may have higher incidence of infectious markers. In addition, saliva from 2 families with 3 and 4 family members was also collected as part of the genetic heritage of *Se* and *se* genes study.

This study was approved by the Ethical Committee of National Blood Centre, The Thai Red Cross Society, Certificate Number NBC 11/2016.

ABO blood grouping

Blood samples were collected at the end of the blood donation process at National Blood Centre, The Thai Red Cross Society. These blood samples were tested for ABO blood groups by cell and serum grouping.[8]

Hemagglutination inhibition test (HAI)

Prior to the HAI test, the working antisera were determined for the optimal dilution by performing 2-fold dilution of anti-A, anti-B and anti-H and tested with corresponding standard A, B and O cells. Then selected for the dilutions which gave 2+ agglutination results.

The inactivated saliva in a volume of 100 μL was taken into 3 test tubes and labelled A, B and H. Antisera A, B and H in the selected dilutions of 1:8, 1:32 and 1:2, respectively, in a volume of 100 μL were added to the corresponding test tubes using 1% BSA in 0.9% NSS to dilute the antisera. The test tubes were mixed and incubated at room temperature for 10 minutes to allow neutralization. Thereafter, 1 drop of 3% corresponding standard cells was added to the test tubes, respectively; mixed and incubated for 30 minutes. The test tubes were then centrifuged and examined for agglutination reactions. For the testing with anti-A, anti-B and anti-H, Se saliva, non-Se saliva and saline were used as the positive, negative and dilution controls, respectively.[8]

Antisera and standard cells were obtained from the Antiserum and Standard Cells Preparation Section, National Blood Centre, The Thai Red Cross Society.

Interpretation

The agglutination reactions for ABO grouping were graded as 4+, 3+, 2+, 1+, w+ and 0. Indication of 4+ represents the strongest agglutination reaction meaning that there is a presence of the corresponding antigen. Indication of 0 represents no occurrence of agglutination meaning that there is no presence of the corresponding antigen.[8]

The agglutination reactions for HAI were graded as 2+ and 0. The negative result (0) indicates the presence of the corresponding ABH substances in the tested saliva, likewise, the positive result (2+) indicates the absence of the corresponding ABH substances.[8]

Statistical analysis was performed using Chi-square and t-tests[9] with a significance level of 5% in order to conclude how significant the influence of gender and ABH blood groups is on the incidence of secretor among the population. The p values of less than 0.05 were considered as significant.

Results

The frequencies of ABO blood grouping were 41.75%, 20.39%, 32.52% and 5.34% for groups O, A,

B and AB, respectively and the gene frequencies *O, A* and *B* were 0.646, 0.142 and 0.212, respectively (Table 1). Secretor and non-secretor status of the study population were 70.87% and 29.13%, respectively (Table 2). In men, 68.00% were secretors, while in women 73.58% were secretors but there were no significant differences between the two genders (χ^2 = 0.778; p = 0.378) (Table 2).

The frequencies of secretor status in different ABO blood groups were 74.42% in O, 64.29% in A, 74.63% in B and 45.45% in AB (Table 3). There were significant differences in the frequency of secretor status in AB blood group when comparing with blood groups O and B (χ^2 = 3.985; p = 0.046 and χ^2 = 3.867; p = 0.049, respectively) but there were no significant differences when comparing with blood group A (χ^2 = 1.292; p = 0.256). However, there were no significant differences in the frequency of secretor status in blood groups O, A and B when compared with each other (all p > 0.05) (Table 4).

A family study had also been done to determine the genetic heritage of the *Se* and *se* genes (Figure 1). The fact that *Se* gene is dominant allowed us to assume that the mother was homozygous *sese* as she

Table 1 Distribution of the blood groups and calculated gene frequencies where *p, q and r* stand for *A, B and O* genes, respectively

	Phenotype frequencies				Gene frequencies		
n	**O**	**A**	**B**	**AB**	***p***	***q***	***r***
206	86 (41.75%)	42 (20.39%)	67 (32.52%)	11 (5.34%)	0.142	0.212	0.646

Table 2 Distribution of secretor status in men and women

Gender	Secretors	Non-secretors	Total
Men	68 (68.00%)	32 (32.00%)	100 (48.54%)
Women	78 (73.58%)	28 (26.42%)	106 (51.46%)
Total	**146 (70.87%)**	**60 (29.13%)**	**206 (100%)**

χ^2 = 0.778; p = 0.378

Table 3 Distribution of secretor status in O, A, B, and AB blood groups

Blood groups	Secretors	Non-secretors	Total
O	64 (74.42%)	22 (25.58%)	86 (41.75%)
A	27 (64.29%)	15 (35.71%)	42 (20.39%)
B	50 (74.63%)	17 (25.37%)	67 (32.52%)
AB	5 (45.45%)	6 (54.55%)	11 (5.34%)
Total	**146 (70.87%)**	**60 (29.13%)**	**206 (100%)**

Table 4 Comparison of frequency of secretor status between blood groups O, A, B and AB

Blood groups and secretor status (%)	O (74.42%)	A (64.29%)	B (74.63%)	AB (45.45%)
O (74.42%)	-	p > 0.05	p > 0.05	p < 0.05
A (64.29%)	p > 0.05	-	p > 0.05	p > 0.05
B (74.63%)	p > 0.05	p > 0.05	-	p < 0.05
AB (45.45%)	p < 0.05	p > 0.05	p < 0.05	-

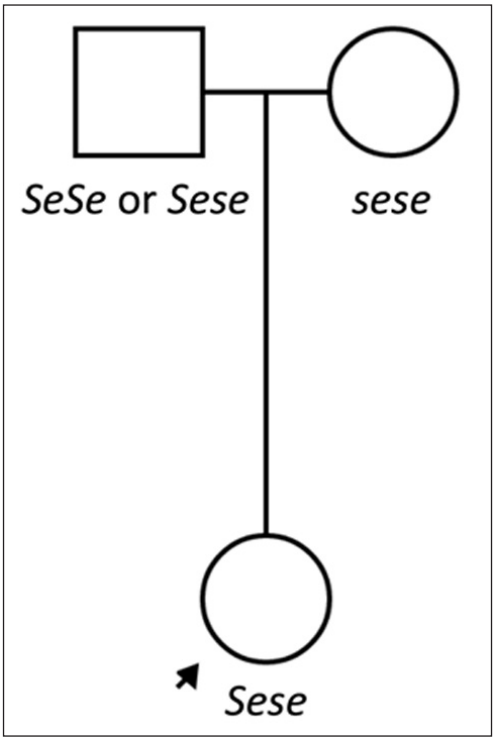

Figure 1 Pedigree showing genetic heritage of the *Se* and *se* genes in one family

was a non-secretor. As the father was a secretor, he was either *SeSe* or *Sese*. Furthermore, the daughter was a secretor and therefore must be *Sese*, carrying *se* gene from the mother and *Se* gene from the father. Unfortunately, everyone in the second family was a secretor, so genetic heritage of *Se* and *se* genes could not be determined.

Discussion

The comparison of the distribution of ABO blood groups and gene frequencies between the Thai population and other populations (Table 5) indicated that all populations acquired a similar pattern, with blood group O being the most frequent and AB being the least frequent. Interestingly, gene frequencies of *A* in Thai (present study) and Indian populations were lower than *B*, while gene frequencies of *A* in Greek and Japanese populations were higher than *B*. However, there were no significant differences in *A* and *B* gene frequencies between these populations (χ^2 = 0.100, p = 0.992 and p > 0.05).[10-12] The distribution of ABO blood groups in the Thai population in this study was similar to those observed by Sringarm S.[13]

Additionally, there were no significant differences when comparing genders to the secretor status (p > 0.05), which was similar to the study reported by Jaff M[2]. There were significant differences in the secretor status in blood group AB comparing with blood groups O and B (both p < 0.05) but there were no significant differences when comparing with blood group A (p > 0.05). Despite this, there were only 11 samples in blood group AB which may influence with these p values. Furthermore, there were no significant differences between blood groups O, A and B and the secretor status when comparing with each other (all p > 0.05). Different studies showed a variation in the secretor status among ABO blood. For instance, one study found that the highest frequency of secretors was in blood group O[2], while another study found that the highest frequency of secretors was in blood group

Table 5 Comparison of the distribution of blood groups and gene frequencies in different populations where *p, q, r* stand for *A, B, O* genes, respectively

Populations	n	Phenotype frequencies				Gene frequencies			Authors
		O	A	B	AB	*p*	*q*	*r*	
Thai	206	86 (41.75%)	42 (20.39%)	67 (32.52%)	11 (5.34%)	0.142	0.212	0.646	Present study
Greek	1105	- (43.73%)	- (38.44%)	- (13.06%)	- (4.77%)	0.245	0.094	0.661	Lialiaris[10]
Bangalore (Indian)	36964	14716 (39.81%)	8817 (23.85%)	11071 (29.96%)	2356 (6.38%)	0.167	0.202	0.631	Periyavan[11]
Japanese	1000	297 (29%)	390 (40%)	213 (21%)	102 (10%)	0.292	0.169	0.539	Maeda[12]

χ^2 = 0.100; p = 0.992; p > 0.05

Table 6 Comparison of the percentage of secretors and non-secretors and gene frequency of *Se* and *se* in various populations

Populations	n	Secretors (%)	Non-secretors (%)	*Se*	*se*	Authors
Thai	206	70.87	29.13	0.4603	0.5397	present study
Northumberland (British)	1752	70.78	29.22	0.4594	0.5406	Mitchell [5]
Southern Britain (British)	5962	76.28	23.72	0.5130	0.4870	Mitchell [5]
Reddis (Indian)	364	75.27	24.73	0.5027	0.4973	Naidu [6]
Kammas (Indian)	131	75.57	24.43	0.5057	0.4943	Naidu [6]

B.[14]

Furthermore, the comparison of frequency of secretors and non-secretors and gene frequencies of *Se* and *se* (Table 6) clearly showed that the status of secretors among the Thai population was comparably similar to other populations.[5,6] Additionally, among the secretors, the ABH secretor status obtained by HAI test corresponded with the results obtained by ABO blood grouping.

A family had been observed in the present study. Figure 1 showed that the daughter was a carrier as her mother was a non-secretor, while her father was a secretor. However, her father could either be *SeSe* or *Sese* as the fate of her paternal grandparents was unknown. Another family had also been studied but everyone in the family was a secretor, therefore the *Se* and *se* genetic heritage could not be illustrated.

Conclusion

The ABH secretion had been elucidated for the first time in the Thai population. Segregation of *Se* and *se* genes was clearly illustrated by family study. In addition, there were no significant differences for *Se* and *se* gene frequencies among genders and in blood groups O, A and B, though there were significant differences when comparing blood group AB with blood groups O and B but no significant differences when comparing with blood group A. This study revealed that frequency of secretors was no different to other populations. The detection of ABH substances in saliva could be used to resolve ABO grouping problem when the routine laboratory test gives inconclusive results.

Acknowledgements

The authors acknowledge the Director of National Blood Centre, The Thai Red Cross Society for the kind permission to allow us to carry out this study, the Head Section of Antiserum and Standard Cells Preparation Section, National Blood Centre, The Thai Red Cross Society for providing us the facilities required and the blood donors for their kind cooperation to enable us to accomplish this study.

References

1. *Dean L. Blood groups and red cell antigens. National Center for Biotechnology Information. Bethesda, MD. 2005.*
2. *Jaff M. Higher frequency of secretor phenotype in O blood group - its benefits in prevention and/or treatment of some diseases. Int J Nanomed 2010;5:901-5.*
3. *Sen MP, Vanishree M, Hunasgi S, Surekha R, Koneru A, Manvikar V. A comparison of absorption inhibition and absorption elution methods for estimation of ABO blood groups in saliva. J Med, Radiol, Pathol & Surg 2015;1:1-4.*
4. *Danzer M, Polin H, Stabentheiner S, Hartmann C, Lennartz K, Gabriel C. Comprehensive polymorphism analysis of ABO using allele-specific separation by bead technology and subsequent sequencing. Vox Sang 2010;98:451-4*
5. *Mitchell RT, Sunderland E. ABH secretion in Northumberland, England. Hum Hered 1982;32:181-4*
6. *Naidu J, Veerraju P, Mukherjee D. ABO blood groups and ABH saliva secretion in Reddis and Kammas Of Southern Andhra Pradesh. Am J Phys Anthropol 1978;48:533-4.*
7. *Kimura A, Matsumura F, Sodesaki K, Tsuji T. ABO blood grouping of saliva from mixed body fluids by sandwich methods using monoclonal antibodies to tissue specific epitopes on blood group substance in saliva. Int J Legal Med 1991;104:189-92.*
8. *Roback JD, Combs MR, Grossman BJ, Hillyer CD, eds. Technical manual. 16th ed. Bethesda, MD : AABB, 2008.*

9. *Mchugh M. The chi-square test of independence. Biochemia Medica 2013;23:143-9.*
10. *Lialiaris T, Digkas E, Kareli D, Pouliliou S, Asimakopoulos B, Pagonopoulou O, et al. Distribution of ABO and Rh blood groups in Greece: an update. Int J Immunogenetics 2011;38:1-5.*
11. *Periyavan S, Sangeetha S, Marimuthu P, Manjunath B, Seema D. Distribution of ABO and Rhesus-D blood groups in and around Bangalore. Asian J Transfusion Sci 2010;4:41.*
12. *Maeda K, Nakamura S, Murakami C, Irie W, Watanabe T, Sasaki C, et al. ABO genotyping by TaqMan assay and allele frequencies in a Japanese population. Leg Med 2013;15:57-60.*
13. *Sringarm S, Chupungart C, Giles C. The Use of Ulex europaeus and Dolichos biflorus extracts in routine ABO grouping of blood donors in Thailand. Vox Sang 1972;23:537-45.*
14. *Saboor M, Ullah A, Qamar K, Mir A, Moinuddin. Frequency of ABH secretors and non secretors: a cross sectional study in Karachi. Pak J Med Sci 2014;30:89-193.*